Experimental Cerebral Edema: Vital Staining with Evans Blue During the Developmental and Regressive Phases*

GEORGE E. BRYAR, M.D., NORMAN P. GOLDSTEIN, M.D., HENDRIK J. SVIEN, M.D., GEORGE P. SAYRE, M.D., AND JAMES D. JONES, PH.D.
Mayo Clinic, Rochester, Minnesota

The genesis of focal edema around tumors in the human is a complex problem and certainly depends on many factors such as expansion of the mass, permeability changes in neovascularized tumor, and perhaps release of toxic substances from the tumor itself. Experimental models in animals can and should be used for the study of this problem, and the psyllium seed model offers distinct advantages over focal freezing. The edema induced by psyllium seed is more closely related to the edema of tumors because of its time and mode of development, its lack of hemorrhage and tissue necrosis, and its response to steroids.

Despite the increasing interest in cerebral edema in the last decade, it remains a formidable clinical and experimental problem. Vital staining has been used to study some forms of cerebral edema for many years, yet its mechanism has never been completely understood and its late phases have only rarely been investigated. In the present investigation, we used vital staining with Evans blue to study the development and regression of experimental cerebral edema produced by the implantation of psyllium seed.

Materials and Methods

Thirty-four full-grown mongrel dogs were used. Each was anesthetized with pentobarbital, intubated, and allowed to breathe room air. Under sterile conditions, a small burr hole was made over the right hemisphere, and the dura was opened. In 31 animals, 0.3 ml of dry, sterilized psyllium seed was implanted through a cannula introduced to a depth of 1 cm beneath the cortex by a modification of the technique of Sperl and associates. No cerebral tissue was removed. In one animal we implanted 0.3 ml of seed that had previously been soaked in sterile saline for 48 hours and allowed to expand. In another animal, 0.3 ml of dry seed was implanted on the right and 0.3 ml of wet, expanded seed was implanted on the left. In still another animal, all the elements of the procedure were performed except the implantation of seed.

An acrylic cranioplasty was performed on the burr hole, and the wound was closed in layers. At the conclusion of the procedure, each dog was given 2.5% solution of Evans blue intravenously (1 ml/kg). The dogs died or were killed with an overdose of pentobarbital 24 hours to 2 years after operation. The brains were removed as quickly as possible, photographed, and divided into two groups: one for morphologic study and one for chemical analysis. In the morphologic group, coronal sections of the formalin-fixed brain were made at 1-cm levels beginning at the implantation site. The sections were photographed and the extent of vital staining was noted. Each piece was embedded in its entirety in paraffin or celloidin and histologic sections were cut. In this way it was possible to compare the treated and untreated hemispheres on the same slide and to correlate the extent of histologically demonstrable edema on the slide with the vital staining visible on the photograph.

In the seven brains in the group for chemical study, the bolus of seed was removed and each hemisphere was weighed and homogenized separately. The Evans blue was extracted from the tissue by a modification of the technique described by Campbell and associates for serum.

Results

All the animals regained consciousness and appeared well by the evening of the day of the operation. Twenty-seven animals lived
until they were killed and were suitable for study. Two of these were controls. Seven animals were unsuitable for study: four were found dead, of unknown causes, in their cages during the first postoperative week; three were excluded because of a moderate amount of cortical bleeding in one, gross infection in another, and rupture of the seed into the ventricle causing hydrocephalus in the third. Thus, edema free of hemorrhage, infection, or significant tissue destruction was produced in 78% of the animals.

**Developmental Phase.** Gross examination showed a sharply circumscribed mass of psyllium seed (Fig. 1), which gradually expanded during the first 4 days (the developmental phase). Changes in the cortex were minimal and were confined to the region immediately adjacent to the seed, in contrast to the striking and progressive changes in the white matter. At 24 hours postoperatively there was softening of the white matter in the vicinity of the seed. This gradually progressed over the next several days to involve regions 2 and 3 cm from the lesion. The gyri were broadened and flattened and the mass of the hemisphere was increased, producing distortion of the midline structures. The white matter felt soft and appeared spongy. The changes were limited to the side of implantation of the psyllium seed; the other hemisphere appeared normal.

Histologically there was tissue destruction and a mild leukocytic response in the region immediately surrounding the seed, but this reaction was limited to the rim of tissue, several millimeters thick, around the seed in both cortex and white matter. In the white matter at a distance from this inflammatory response, the fibers were pale and separated by amorphous, periodic acid-Schiff (PAS)-positive fluid. By the end of the fourth day this reaction involved much of the white matter in the treated hemisphere (Fig. 2). The astrocytes were enlarged and had prominent PAS-positive cytoplasm. Cajal's gold chloride stain showed enlargement of both the cell bodies and the processes (Fig. 3). These changes were most marked in the region of the seed and diminished with increasing distance from the lesion. The opposite hemisphere appeared normal.

The mass of seed stained intensely with Evans blue. The cortex and white matter immediately surrounding the seed stained moderately in a distribution corresponding to the region of inflammation seen histologically. The vital staining of the white matter corresponded to the regions of morphologic edema. In the cortex there was a small area of staining surrounding the lesion but little demonstrable edema.

The animals which had had a sham operation or the implantation of expanded wet seed were killed on the fourth day. Their brains showed a mild inflammatory response surrounding the seed or the cannula tract. There had been no enlargement of the bolus of seed. There was mild staining with Evans blue limited to the 2 to 3 mm immediately surrounding the seed, but there was no spread of dye from this area and no histologic evidence of edema.

**Peak Intensity Phase.** During this phase (days 4 through 7), the mass of seed reached its maximal size and remained sharply circumscribed. The gross changes in the white matter were similar to those of the previous phase but were more widespread. The cortex appeared normal. In five animals killed on the fourth and fifth days, the weight of each hemisphere was determined after removal of the seed. The edematous hemispheres were 7.6% to 18.4% (mean, 11.7%) heavier than the opposite (control) hemispheres.

Histologically the inflammatory response in the region immediately surrounding the seed was subsiding at this stage. The pallor and the separation of the fibers in the white matter were more widespread and a greater amount of fluid was present. There was a relative sparing of the arcuate fibers and the corpus callosum. The astrocytes continued to be prominent.

The seed had lost some of the blue coloration and it began to appear brownish yellow. Except for the region immediately surrounding the seed, the cortex was not stained with Evans blue. However, the white matter was stained a deep blue up to several centimeters from the lesion. The region of vital staining exceeded the region of morphologically demonstrable edema by the end of 1 week.

**Regressive Phase.** As the regressive phase